

MINIREVIEW

Transfer of Antibiotic Resistance Genes between Gram-Positive and Gram-Negative Bacteria†

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INTRODUCTION

Four major mechanisms account for the evolution of bacterial resistance to antibiotics that correlates with the use of the drugs: (i) emergence of "new" opportunistic pathogenic soil microorganisms that are often multiresistant to antibiotics (e.g., *Acinetobacter* spp.); (ii) emergence of "new" acquired resistance mechanisms (e.g., glycopeptide resistance in enterococci); (iii) occurrence of mutations in genes located in the host chromosome (e.g., encoding DNA gyrase) or plasmid borne (e.g., for extended-spectrum β -lactamases); and (iv) spread of "old" (i.e., already known) resistance genes into "new" bacterial hosts (i.e., genera or species that were previously uniformly susceptible). The last mechanism has been known since the early finding that antibiotic resistance genes are often part of self-transferable plasmids or of transposable elements. However, and until recently, it was thought that this type of genetic transfer only occurred between closely related bacteria (1, 24). This review will focus on two recent notions: (i) the transfer of antibiotic resistance genes in natural environments can occur between phylogenetically distant bacterial genera, in particular between gram-positive and gram-negative bacteria; and (ii) conjugation is a mechanism of transfer of genetic information with a very broad host range.

INDIRECT EVIDENCE FOR NATURAL GENE TRANSFER BETWEEN GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Elements for the inference of horizontal genetic transfer between distantly related bacteria. That horizontal gene transfer has occurred under natural conditions is difficult to prove. Nevertheless, observations at various biological levels allow, in certain cases, the deduction of lateral gene flow.

(i) **Resistance phenotype.** Careful examination of antibiotic resistance phenotypes may provide circumstantial evidence of gene transfer (4, 17). The detection of a new resistance phenotype in a bacterial genus or species suggests the acquisition of foreign genetic information. However, this suggestion can be misleading, since coexistence in the same host of different mechanisms can phenotypically mimic another, single mechanism. Nevertheless, this preliminary step constitutes a prerequisite for further studies.

(ii) **Resistance mechanism.** When easy to assess, as in the case of certain antibiotic-modifying enzymes, the mechanism

of resistance can provide an additional clue as to intergeneric transfer (17).

(iii) **Resistance gene or protein.** Confirmation of horizontal gene flow comes from the determination and comparative analysis of the sequences of the resistance gene and of its deduced product. When gene transfer between gram-positive and gram-negative bacteria is suspected, strong cases can be made because of the availability of sequence data from a variety of microorganisms. The information required for deducing such an event includes (i) the sequence of the transferred gene from various adequately divergent gram-positive and gram-negative bacterial genera and (ii) a comparable set of data from the same or closely related organisms for another gene(s). In fact, the majority of the possible resistance gene transfers suggested between gram-positive and gram-negative bacteria are based on the finding of an unusually high level of similarity among homologous sequences from distantly related genera (Table 1). Recently, a nonparametric method was proposed to test whether these inconsistencies in taxonomic relationships implicit in different sets of nucleic acid sequences resulted from the horizontal transfer of genetic material between taxa rather than from convergent evolution or variations in evolutionary rates (18).

Tracing the origin of resistance genes. If the notion of horizontal gene flow relies essentially on the lack of congruence between resistance gene sequence identity and the divergence time between the host bacteria, several lines of evidence allow tracing of the origin of the resistance gene: (i) the high level of sequence similarity among resistance determinants in clinical isolates of the putative donor species from diverse geographical locations (28); (ii) the abundance of the resistance determinant in strains of the possible donor relative to its rarity in the recipient (8, 17, 28, 29); (iii) the existence of ecosystems shared by the donor and the recipient, such as the digestive tract (11); (iv) the structure of the resistance gene, notably the primary structure of the promoter (35, 44), the base composition of its open reading frame relative to that of the genomic environment (3, 8), and codon usage compared with that of the host chromosome (3, 8, 22, 35, 44); (v) the existence of barriers to heterologous expression (in particular, the fact that genes from gram-positive microorganisms are readily expressed in gram-negative bacteria, whereas the reverse is generally not true [20], exerts a strong directional selection); and (vi) the possibility of back-transfer of the gene, under laboratory conditions, to its putative progenitor followed by expression studies at the level of transcription (8, 44) or of the resistance phenotype (8, 44). On the basis of all these criteria for the *aphA-3* and *ermB* genes and for some of them for the other resistance determinants listed in Table 1, there is a gene flux in nature from gram-positive cocci, and most probably from enterococci and streptococci, to gram-negative

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† This review is dedicated to the memory of Jean-Claude Clément.

TABLE 1. Inferred natural antibiotic resistance gene transfer from gram-positive cocci to gram-negative bacteria

Gene	Resistance phenotype ^a	Original host genus	New host	Reference(s)
<i>aphA-3</i>	Am, Km	<i>Enterococcus</i> , <i>Streptococcus</i> , <i>Staphylococcus</i>	<i>Campylobacter coli</i>	17, 44
<i>aadE</i>	Sm	<i>Enterococcus</i> , <i>Streptococcus</i> , <i>Staphylococcus</i>	<i>Campylobacter</i> spp.	29
<i>ermB</i>	MLS	<i>Enterococcus</i> , <i>Streptococcus</i> , <i>Staphylococcus</i>	<i>E. coli</i> , <i>K. pneumoniae</i>	2, 4, 8
<i>ermC</i>	MLS	<i>Staphylococcus</i>	<i>E. coli</i>	21
<i>tet(M)</i>	Tc, Mc	<i>Enterococcus</i> , <i>Streptococcus</i> , <i>Staphylococcus</i>	<i>Eikenella corrodens</i> ^b , <i>Fusobacterium nucleatum</i> , <i>Gardnerella vaginalis</i> , <i>Haemophilus</i> spp., <i>Kingella denitrificans</i> , <i>Neisseria</i> spp.	30
<i>tet(O)</i>	Tc, Mc	<i>Enterococcus</i> , <i>Streptococcus</i>	<i>Campylobacter</i> spp.	35, 47
<i>ereB</i>	Em	ND ^c	<i>E. coli</i>	4

^a Am, amikacin; Em, erythromycin; Km, kanamycin; Mc, minocycline; MLS, macrolides-lincosamides-streptogramins; Sm, streptomycin; Tc, tetracycline.

^b *tet(M)* was also found in *Ureaplasma urealyticum* and *Mycoplasma hominis* (30).

^c ND, not determined.

bacteria. It also appears that the gene flux with this polarity is recent and extensive, such that the *tet(O)* gene was first detected in *Campylobacter* spp. and predicted from structural features to originate from gram-positive cocci (35), in which it was later found (47). The original gram-positive host of the *ereB* gene has not yet been found (3). However, this determinant confers a narrow resistance phenotype to 14-membered macrolides only, is often associated with and masked by *erm* genes that confer broad resistance phenotypes, and is thus difficult to trace.

trans-GRAM CONJUGATION

As already mentioned, antibiotic resistance determinants from gram-positive organisms are readily expressed in gram-negative bacteria. Thus, the only barrier to the acquisition of genes from a gram-positive bacterium by a gram-negative bacterium lies in the transfer process itself and in the stable replication of exogenous DNA. Three DNA transfer processes can conceptually account for the gene flux from gram-positive to gram-negative bacteria. However, their probabilities of occurrence in nature differ greatly. Transfer of genetic information by transduction between gram-positive and gram-negative organisms has never been obtained under laboratory conditions. Transformation of short blocks of DNA, usually a few hundreds of base pairs in size, leads to antibiotic resistance genes with a mosaic structure, such as those for hybrid penicillin-binding proteins in penicillin-resistant *Streptococcus pneumoniae* and *Neisseria* spp. (36). In addition, this natural transfer occurs only between related species that are sufficiently similar (less than 25% difference in DNA sequence) for homologous recombination to occur (36). Gene transfer from gram-positive to gram-negative bacteria was detected in species that show natural transforming ability (Table 1), but structural analysis indicated the transfer of resistance genes en bloc rather than of portions of genes (17, 35, 44, 47). Although these observations do not rule out the possibility that *trans*-gram transfer by transduction or transformation can occur under natural conditions, an impossible claim to substantiate, we tested the possibility that the exchange of genetic material between distantly related species was mediated by conjugation (38).

trans-Gram plasmid conjugation. (i) **Transfer from gram-positive to gram-negative bacteria.** Since, apart from a few exceptions (5, 12, 14, 33, 45), plasmids from gram-positive bacteria cannot be stably maintained in gram-negative organisms, the possibility of transfer of genetic information by conjugation from gram-positive to gram-negative bacteria was studied with a hybrid bireplicon (38). The shuttle vector contained the transfer functions and the origin of replication of a broad-host-range enterococcal plasmid, the origin of replication of an enterobacterial plasmid, and a resistance gene, *aphA-3*, known to be expressed in both gram-positive and gram-negative bacteria. This chimeric plasmid was successfully transferred repeatedly, albeit at a low frequency, by conjugation from *Enterococcus faecalis* to *Escherichia coli* on solid medium. Enterococci and streptococci, members of the family *Enterobacteriaceae*, and *Campylobacter* spp. share numerous human colonization sites, in particular the gastrointestinal tract. Since the latter ecosystem is the most probable meeting point for these bacteria in nature, transfer of the same plasmid between *E. faecalis* and *E. coli* in the digestive tract of gnotobiotic mice was also attempted. Conjugal transfer was also obtained in vivo and in the absence of selective pressure (11). The animal model used is probably more favorable for genetic exchange by conjugation than are natural conditions, since it allows intestinal colonization by large numbers of donors and recipients. However, it mimics the situation prevailing in the guts of antibiotic-treated human beings and animals, in whom suppression of the indigenous intestinal microflora allows efficient colonization with nonenteropathogenic strains of members of the family *Enterobacteriaceae*. Conjugation is therefore a mechanism that could account for the antibiotic resistance gene flux observed in nature and, in particular, for the presence of the enterococcal-streptococcal erythromycin resistance gene *ermB* in members of the family *Enterobacteriaceae* isolated from patients treated orally with this antibiotic (2).

(ii) **Transfer from gram-negative to gram-positive bacteria.**

(a) **Shuttle plasmids.** Conjugative plasmids belonging to incompatibility groups N, P, Q, and W have a very broad host range and can be transferred to a large variety of gram-negative bacterial species (37). The host range of a self-transferable plasmid depends upon the host range of its

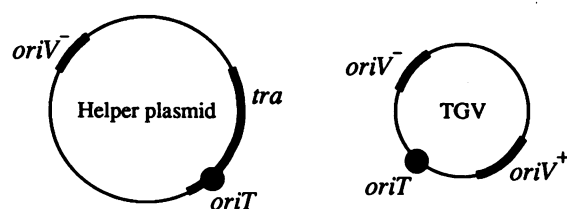


FIG. 1. Plasmid content of an *E. coli* mobilizing strain. *oriT*, origin of conjugal DNA transfer; *oriV⁻*, origin of vegetative replication active in gram-negative bacteria; *oriV⁺*, origin of vegetative replication active in gram-positive bacteria; *tra*, transfer region with *trans*-acting mobilization function; TGV, *trans*-gram vector or mobilizable shuttle plasmid.

conjugation system (i.e., the range of genera and species into which it can be conjugally transferred irrespective of its stable inheritance by the progeny of the new host) combined with that of its replication system. On the basis of the observation that IncP plasmids can be transferred to bacterial species in which they cannot replicate (37), a series of mobilizable shuttle vectors that are transferable from *E. coli* to various gram-positive bacteria by conjugation was designed (39–41). The modules in these recombinant plasmids consist of the replication origins of a broad-host-range enterococcal plasmid and of an enterobacterial plasmid, the transfer origin of an IncP plasmid, and a resistance gene expressed in both gram-positive and gram-negative organisms. In this binary system (Fig. 1), mobilization of these vectors from *E. coli* to gram-positive bacteria is due to the presence in the donor cells of a coresident self-transferable IncP helper plasmid (39). Vectors of this type were transferred by mating on solid medium to a large variety of gram-positive hosts (Table 2). The results suggest that genetic exchange from gram-negative to gram-positive bacteria could occur in nature as it does from gram-positive to gram-negative organisms (Table 1). The mobilization process requires the presence of an origin of transfer in *cis* and of adequate *tra* functions in *trans* (38). The low frequency of transfer ($\leq 10^{-8}$) observed with certain species could be due to the presence of a restriction system in the recipient cells (31). This finding is surprising, since it is generally considered that conjugation consists of the transfer of single-stranded DNA that is insensitive to the majority of restriction systems. Protective DNA methylation in the recipient bacterium may not occur immediately after synthesis of the complementary strand, allowing restriction of the incoming, newly synthesized double-stranded DNA. This barrier to plasmid mobilization from *E. coli* to gram-positive bacteria can be overcome by heat treatment of the recipient cells prior to mating (31). The cell wall of gram-positive bacteria also constitutes an important barrier for conjugative transfer of genetic information delivered from *E. coli*, since the presence of subinhibitory concentrations of penicillin in the mating medium results in a significant increase in transfer frequency (43).

The recent discovery of the extraordinarily wide host range of the conjugative process is of interest not only with respect to the dissemination of resistance genes in the environment but also from a biotechnology point of view, since it includes medically important bacterial genera, such as *Mycobacterium*, and industrially important bacterial genera, such as *Clostridium* and *Streptomyces* (Table 2). Genetic analysis and manipulation of gram-positive bacteria have been greatly facilitated by the development of transformation (or transfection) systems, whether natural or induced by chemical or physical treatments. However, few species (*Bacillus subtilis*, *Streptococcus*

TABLE 2. *trans*-Gram conjugation under laboratory conditions

Transfer		Vector	Reference(s)
From	To		
<i>E. faecalis</i>	<i>E. coli</i>	Shuttle plasmid ^a	11, 38
	<i>Alcaligenes eutrophus</i> , <i>Citrobacter freundii</i> , <i>E. coli</i>	Conjugative transposon ^b	6
<i>E. coli</i>	<i>Bacillus</i> spp., <i>E. faecalis</i> , <i>Listeria</i> spp., <i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp.	Shuttle plasmids	39–43
	<i>Clostridium acetobutylicum</i>	Shuttle plasmids	46
	<i>Streptomyces</i> spp.	Shuttle plasmids	7, 25, 34
	<i>M. smegmatis</i>	Shuttle plasmids	19
	<i>Arthrobacter albidus</i> , <i>Brevibacterium</i> spp., <i>Corynebacterium</i> spp.	Shuttle plasmid	31
	<i>M. smegmatis</i> , <i>S. lividans</i>	Natural plasmid	13
	<i>B. subtilis</i> , <i>Clostridium acetobutylicum</i> , <i>E. faecalis</i> , <i>Streptococcus lactis</i>	Conjugative transposon	6
	<i>Bacillus stearothermophilus</i>	Conjugative transposon	27

^a Transfer was obtained under laboratory conditions and in the digestive tracts of gnotobiotic mice.

^b The fact that conjugation was promoted by the transfer functions of the transposon is questionable (see the text).

mutans, *S. pneumoniae*, and *Streptococcus gordonii*) are naturally competent, and protoplast transformation is time- and labor-intensive and neither efficient nor reproducible. Conjugation thus represents a general and versatile transfer system for genetic engineering in a wide range of gram-positive bacteria, and numerous cloning (7, 40, 41) and integrative (34, 42) vectors have already been developed.

(b) **Natural plasmids: return to sender?** In subsequent experiments (13), plasmid RSF1010, of incompatibility group Q, was successfully mobilized from *E. coli* to *Streptomyces lividans* and *Mycobacterium smegmatis* (Table 2). That this plasmid could replicate and was stably maintained in its new hosts may have resulted from its particular mode of replication which, as opposed to those of most other extrachromosomal replicons, does not require host-encoded replication functions (32). The high G+C content (61%) of this promiscuous plasmid, which is very similar to those of mycobacteria and *Streptomyces* spp., combined with the phenotypic expression of its two resistance determinants in the new hosts, may well indicate that, in fact, this plasmid originates from these gram-positive microorganisms.

***trans*-Gram transposon conjugation.** Conjugative transposon Tn916 was found to be transferred from *E. coli* to a variety of gram-positive recipients (Table 2), in which it was trans-

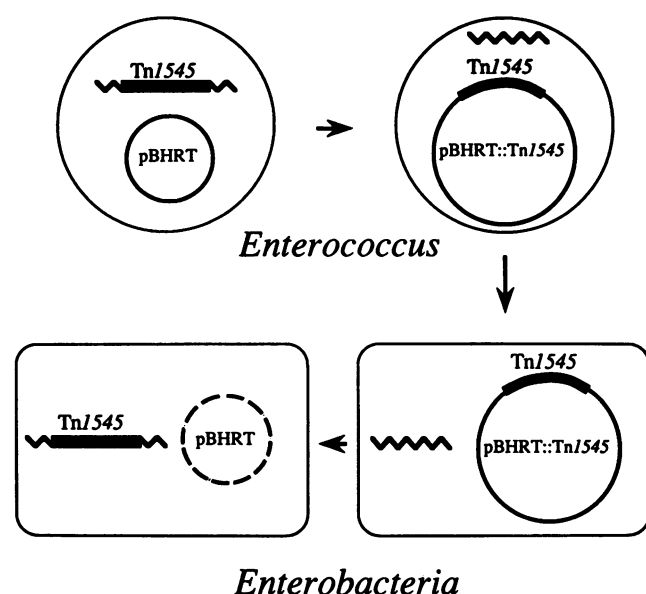


FIG. 2. Proposed mechanism for gene transfer between gram-positive cocci and gram-negative bacilli. Wavy line represents chromosomal DNA. pBHRT is a plasmid with a broad host range for transfer.

posed into the chromosome. The opposite transfer was also obtained. However, in the latter experiments (6), Tn916 was borne by a self-transferable plasmid in the donor; thus, one could not determine whether conjugation was due to the transfer function of the transposon, the plasmid, or a combination of both.

Proposed model for trans-gram gene transfer. Analysis with the recipient of the genetic basis (17, 47) and of the genomic environment (3, 8) of the acquired foreign DNA indicated that extension of the gene pool of gram-positive cocci to gram-negative bacteria results from an acquisition of resistance genes rather than of replicons en bloc. This observation is consistent with the notion that, as already mentioned, in most instances plasmids from gram-positive bacteria cannot be stably inherited in gram-negative bacteria. Therefore, efficacy of gene transfer from a gram-positive bacterium to a gram-negative bacterium in nature results from the combined multiplicative probabilities of the transfer process and of the mechanism for the stabilization of exogenous DNA. The most parsimonious and probable mechanism of transfer of genetic information under these constraints is conjugation followed by integration, upon entry, of the foreign DNA into the genome of the new host (Fig. 2). The absence of homology between the genomes of phylogenetically remote bacteria makes illegitimate recombination a likely step for the in vivo stabilization of exogenous DNA in prokaryotes. Resistance gene flux from gram-positive to gram-negative bacteria is therefore probably facilitated by the presence of genes on transposable elements. The finding of resistance genes *ermB*, *aphA-3*, and *tet(M)* on enterococcal-streptococcal transposons, which are active in gram-negative bacteria (9, 10, 15, 16), constitutes further support for this notion. The outcome of the two successive genetic events, conjugation and transposition, could have been obscured in part by the molecular rearrangements observed in the new hosts (8, 44). This genomic remodeling probably occurred during the process of conjugal transfer (38) or during the subsequent spread among gram-negative bacteria or was intended for selective expression in the transconjugants (8).

CONCLUSION: trans-GRAM PROMISCUITY

Antibiotic resistance determinants constitute a privileged system for the study of naturally occurring DNA transfer among bacteria, because they are easy to trace and because of the massive selective pressure exerted by antibiotic use for human therapy and animal feeding. The finding of inconsistencies in molecular data (i.e., the quasi-identity of a resistance determinant from gram-positive and gram-negative bacteria and the lack of congruence between this high degree of similarity and the divergence time between the two groups of bacteria) led to the notion of horizontal gene flux between these distantly related microorganisms. In addition, there is accumulating inferential evidence that this gene transfer is recent, extensive, and polar; i.e., gram-negative bacteria have access to the gene pool of gram-positive cocci. It is thus most unfortunate that current trends in antibiotic therapy, in particular the use of quinolones and oral cephalosporins, tend to select bacteria, i.e., enterococci and streptococci, that act as the reservoir of resistance genes in nature. Conjugation, because of its extraordinarily broad host range, combined with transposition is the most likely mechanism by which genes are transferred between reproductively isolated bacterial species. Lateral gene transfer, erroneously designated bacterial sex (23), is of obvious importance for species evolution (26). This recently recognized phenomenon is of general interest, since it applies to bacteria that are medically and industrially important but also to genetically engineered microorganisms.

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